The Anatomy and Functional Morphology of the Reproductive System of *Bulla gouldiana*[1]

(Gastropoda : Opisthobranchia)

BY

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(2 Plates; 8 Text figures)

INTRODUCTION

Hermaphroditism characterizes opisthobranch reproductive systems, and in opisthobranchs a complex series of ducts, folds and grooves have evolved to achieve separation of sexual products. Some gastropods, including nudibranchs, have attained complete separation of the vas deferens and oviduct to prevent self-fertilization and mixing of the gametes (Lang, 1896; Chambers, 1934). Most primitive tectibranchs, however, do not achieve these divisions and possess incompletely divided ducts. These channels for oocyte and sperm transport are often separated only by a groove or fold of tissue, and frequently all of the gametes must pass simultaneously through these tracts.

Previous studies on opisthobranchs have concentrated on the anatomical detail of these complex reproductive systems. Early workers, including Cuvier (1803), Garstang (1889-1890), Mazzarelli (1891), Lang (1896), Pelseneer (1896) and Guiart (1901) described a variety of opisthobranch reproductive systems with an emphasis on the anatomy and histology. More recent studies concern the anaspids. Species of *Aplysia* have been investigated by Eales (1921; 1938), Winkler (see Beeman, 1970), Beeman (1968a), and Thompson & Bebbington (1969). Beeman (1970) presented an account of the anatomy, histology and functional morphology of *Phyllaplysia taylori* and contributed much to the understanding of anaspid reproduction. The nudibranchs have also received attention, and descriptions of their functional anatomy are given by Chambers (1934), McGowan & Pratt (1954) and Thompson (1966; 1969).

Reproduction in the primitive order Cephalaspidea has been studied in several species. Marcus (1957) and Tchang-Si (see Marcus, 1957) described the reproductive system of *Bulla gouldiana* but did not discuss details of the female gland mass. Fretter (1960) and Fretter & Graham (1954) discussed reproduction in *Ringicula* and *Acteon* respectively; and Brown (1934), Vayssière (1879-1880), Perrier & Fischer (1911), Wells & Wells (1962), Risbec (1951), Johannson (1954), Pruvot-Fol (1954; 1960), Marcus & Marcus (1956), Lloyd (1952), Hyman (1967), and Tchang-Si (1934) considered reproduction in a variety of other cephalaspideans. Lemche (1956) presented an excellent review of the reproductive anatomy and functional morphology in several species of *Cylichna*.

One of the largest and most abundant Pacific Ocean cephalaspideans is *Bulla gouldiana* (Pilsbry, 1893), or Gould's bubble shell. This snail inhabits intertidal mudflats from Santa Barbara, California, to Baja California, Mexico (Allen, 1969). Only a few biological studies have been concerned with *Bulla gouldiana*. Bergh (1901) described various systems in the Bullidae and included descriptions of the male copulatory apparatus of *B. nebulosa*, later referred to as *B. gouldiana* by Marcus (1961). Collective works on Pacific marine animals include short descriptions of the shell, habitat, feeding behavior, and egg mass of *B. gouldiana* (Ricketts & Calvin, 1968; Abbott, 1954; Johnson & Snook, 1927; MacGinitie & MacGinitie, 1949; Allen, 1969). Paine (1963) discussed locomotory and burrowing behavior and the preda-

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tory relationship involving *B. gouldiana* and *Navanax inermis* Cooper, 1862, and Ghiselin (1965) mentioned the penis and prostate gland and the formation of the spermatic bulb in *B. gouldiana* in his work on the phylogeny of opisthobranchs.

In this study the anatomy, histology and functional morphology of the reproductive system of *Bulla gouldiana* are described. No other previous work has, as far as I have been able to ascertain, provided a complete description of the reproductive anatomy of this common Pacific species.

MATERIALS AND METHODS

Bulla gouldiana was collected in the San Diego flood control channel (32°46′N; 117°15′W), San Diego, California. Specimens were taken at night during low tide and were brought to the laboratory and kept in aerated sea water until further examination.

Prior to dissection animals were relaxed by immersion for 1 hour in magnesium chloride solution isotonic with sea water (Beeman, 1968b; 1970) or frozen and then thawed to room temperature. Portions of the system including the ampulla, ovotestis, and contents of the lumina of several glands were transferred to glass slides and examined with a compound microscope for comparison with fixed preparations.

After observations of the gross anatomy, the reproductive structures located in the posterior body section were removed, fixed in Bouin's solution overnight, and then transferred to 70% alcohol (Figures 1, 2). After removal of all the picric acid from the organs several drops of methyl green stain were added to the 70% ethanol containing the structures and the mixture was left for 24 hours (Beeman, 1970). Methyl green stains mucus secreting areas, and the locations of the mucous glands in the female gland mass and common genital duct were easily determined using this procedure.

For histological study, the reproductive organs were fixed for 24 hours in Bouin's solution and transferred to 70% ethanol. The fixed tissues were then dehydrated in alcohol, cleared in toluene and embedded in paraffin (Weesner, 1960). The tertiary butyl alcohol and paraffin oil method described by Weesner (*op. cit.*), which prevents excessive hardening of delicate tissues, was used for studying the female gland mass which became brittle in the alcohol-toluene series. Tissue sections were cut from 8 to 10 μm, and were stained with Mayer's hematoxylin or with Mallory-Heidenhain (Humason, 1967).

Some tissues were fixed in Karnovsky's fixative (Karnovsky, 1965), post-fixed in 2% OsO_4, dehydrated in alcohol, and embedded in Araldite. Thick sections were taken at 1 μm on a Porter-Blum MT-2B ultramicrotome and stained with methylene blue-azure II.

The functional morphology of the reproductive system was examined in the field and laboratory. In the field, observations were made in the flood control channel at night during low tide. Copulation and oviposition were observed, and afterward some of the animals were plunged into liquid nitrogen. All reproductive processes were stopped immediately, and in the laboratory dissections were performed on the frozen specimens that had thawed to room temperature.

RESULTS

Many names and functions have been assigned to opisthobranch reproductive structures summarized by Pruvot-Fol (1960). Ghiselin (1965) re-examined these struc-

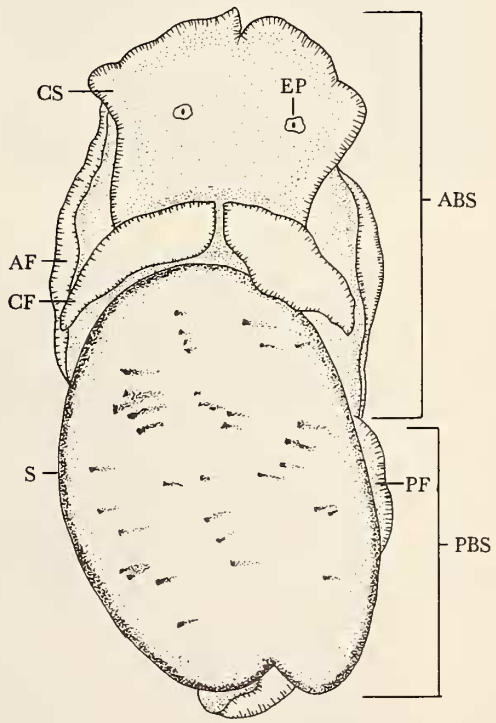

Figure 1

Diagram showing main external features of *Bulla gouldiana*

ABS – Anterior body section
AF – Anterior foot
CF – Cephalic flap
CS – Cephalic shield
EP – Eyespot
PBS – Posterior body section
PF – Posterior foot
S – Shell

tures and their functions in a multitude of opisthobranchs, and presented a classification of these mollusks based on the functioning of their reproductive systems. In the present study Ghiselin's nomenclature is used.

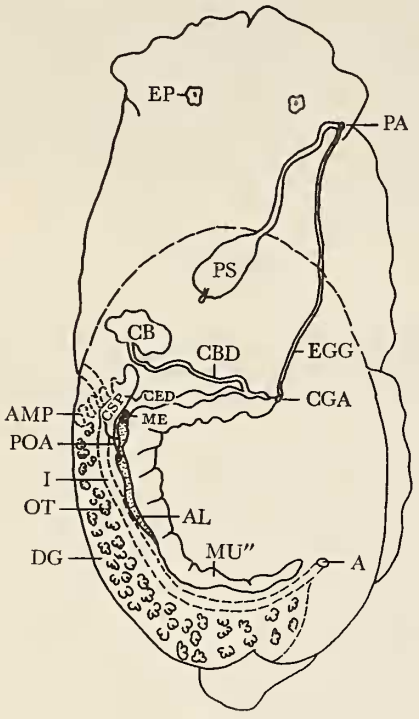

Figure 2

Dorsal view of general body structure showing internal placement of reproductive organs approximately × 2

A – anus
AL – Albumen gland
AMP – Ampulla proper
CBD – Bursa copulatrix duct
CB – Bursa copulatrix
CGA – Common genital aperture
CSP – Common genital duct, comma-shaped portion
CGD – Common genital duct
DG – Digestive gland
EGG – External genital groove
EP – Eyespot
I – Intestine
ME – Membrane gland
MU" – Mucous gland, secondary lobe
PA – Penial aperture
PS – Penial sheath
POA – Post-ampullar duct
OT – Ovotestis

General Body Organization

Adult *Bulla gouldiana* range in length from approximately 2 to 6 cm. Their bodies are organized into anterior and posterior sections which are covered dorsally by a thin shell (Figures 1, 2). The anterior body section is composed of the cephalic shield and flaps, the anterior section of the foot, and a thick, muscular body wall that connects the head and foot laterally. Within the anterior section is the hemocoel which contains the buccal cavity, esophagus, crop, and most of the nervous system. The penial sheath, which encloses the penis and prostate gland, lies above and is sometimes entwined around the buccal mass.

The posterior region of the body is separated from the anterior section by a muscular wall, and is covered dorsally and laterally by the shell. The posterior region contains the remainder of the body organs and the bulk of the reproductive system, including the ovotestis and pallial and coelomic gonoducts (Figure 2).

Ovotestis

Intermingled among the greenish brown digestive gland lobules is the hermaphroditic gonad, the ovotestis (Figures 2, 3). During the spring and summer the ovotestis appears as numerous, irregularly shaped yellow-orange patches situated in the posterior region of the digestive gland. In the late fall and winter months these patches become less conspicuous.

Closer examination shows that the patches consist of many acini, or follicles, each separately bounded by a connective tissue sheath (Figures *4*, *5*). The outer portion of each acinus is bulbous, with the inner region narrowed to form a short ductule. This ductule empties, with ductules from adjacent acini, into the pre-ampullar ducts.

Both spermatogenesis and oogenesis occur within each follicle. Oogenesis occurs in the outer region of the acinus, while spermatogenesis is limited to the inner portion (Figure *4*).

Explanation of Figures *4* to *6*

Figure *4*: Cross-section through ovotestis showing arrangement of spermatogenic and oogenic cells × 80
Figure *5*: Cross-section through mature oocyte in acinus × 130
Figure *6*: Cross-section through ampulla proper in region of columnar ciliated strip × 80

AC – Acinus
OV – Oocyte, mature
CC – Columnar ciliated cell
SP – Spermatozoa

Figure 4

Figure 5

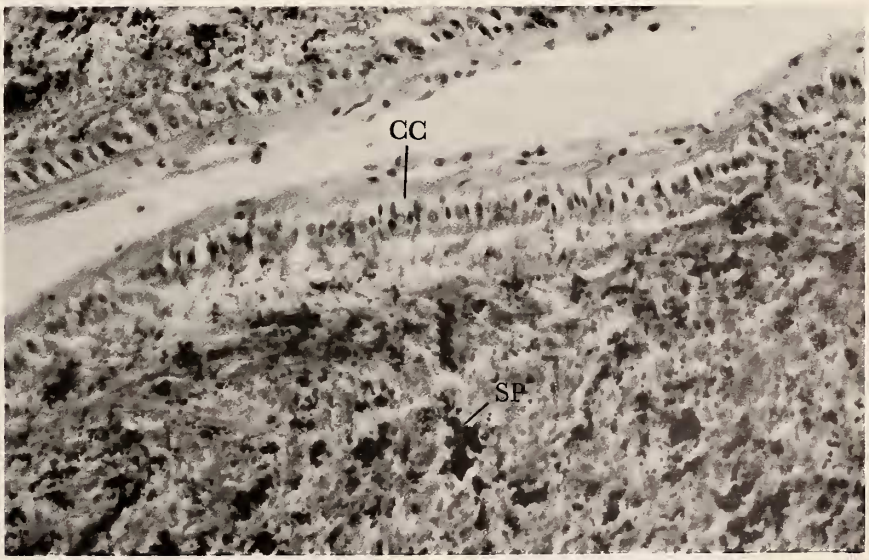

Figure 6

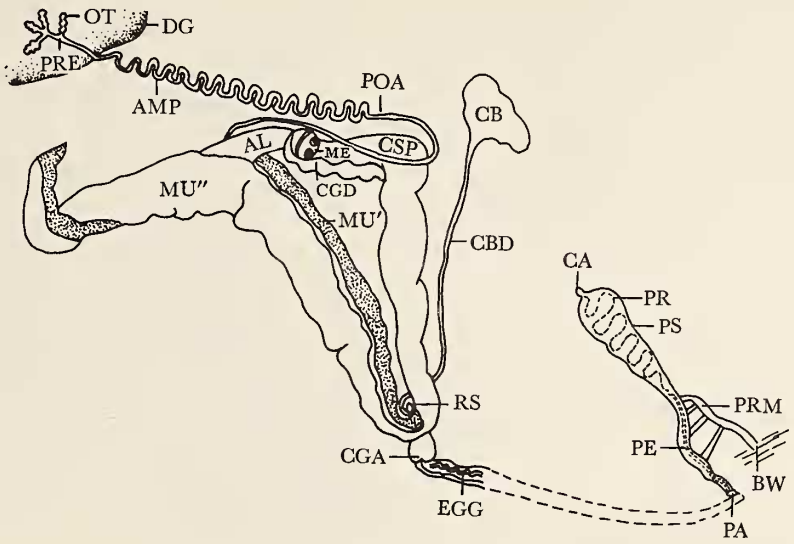

Figure 3

Dorsal view of reproductive system with mucous gland and common genital duct separated. The external genital groove is shortened to include the copulatory apparatus in the diagram. The positions of the penis and prostate gland in the penial sheath are indicated by a dashed line approximately × 6

AL – Albumen gland
AMP – Ampulla proper
BW – Body wall
CA – Caecum
CB – Bursa copulatrix
CBD – Bursa copulatrix duct
ME – Membrane gland
MU' – Mucous gland, primary lobe
MU" – Mucous gland, secondary lobe
OT – Ovotestis
PA – Penial aperture
PE – Penis
CGA – Common genital aperture
CGD – Common genital duct
CSP – Common genital duct, comma-shaped portion
DG – Digestive gland
EGG – External genital groove
RS – Bursa copulatrix seminal reservoir
POA – Post-ampullar duct
PR – Prostate gland
PRE – Pre-ampullar ducts
PRM – Penial retractor muscle
PS – Penial sheath

Coelomic Gonoduct

The coelomic gonoduct, often referred to as the hermaphroditic duct, is located between the gonad and the fertilization chamber (Beeman, 1970). This long continuous tube is divided anatomically into 3 regions including the pre-ampullar duct, the ampulla proper, and the post-ampullar duct.

Pre-ampullar duct: The acini of the ovotestis empty into the pre-ampullar portion of the coelomic gonoduct which consists of many collecting ducts converging from the numerous acini throughout the ovotestis (Figure 3). Eventually the pre-ampullar ducts join to form larger ducts, and at the ventral surface of the first and most dorsal whorl of the digestive gland these ducts emerge to form the ampulla proper.

The pre-ampullar ducts consist of a single layer of squamous cells surrounded by a few muscle and connective tissue fibers.

Ampulla proper: The ampulla proper surfaces on the ventral side of the digestive gland and continues beneath the digestive gland and female gland mass towards the fertilization chamber (Figures 2, 3). Connective tissue extends laterally and anchors the convoluted ampulla to the first shell whorl that lies immediately beneath the duct. As the ampulla approaches the comma-shaped region of the common genital duct, it ends as a narrow constriction.

Approximately $\frac{2}{3}$ of the lumen of the ampulla is lined with a layer of squamous cells, and the remaining $\frac{1}{3}$ is covered with a strip of columnar ciliated cells (Figure 6). The entire duct is surrounded by a layer of connective tissue. When the ampulla narrows near the common genital duct, the entire lumen becomes lined with columnar ciliated cells.

Post-ampullar duct: Following the ampulla, the coelomic gonoduct continues as the post-ampullar duct. This duct passes between the common genital duct and the copulatory bursa and then turns posteriorly and continues dorsally over the membrane and albumen glands (Figures 2, 3, 7). The post-ampullar duct is firmly secured to the surface of these glands by connective tissue. Over the

albumen gland the post-ampulla bends 180° anteriorly and after a short distance disappears into the female gland mass. Within the mass the post-ampulla continues through the anterior region of the albumen gland, and ends at the fertilization chamber near the origin of the membrane gland and the beginning of the pallial gonoduct.

Histologically, the post-ampulla is divided into 2 regions. The first region includes the duct from its origin at the end of the ampulla proper to the anterior bend over the dorsal surface of the albumen gland. This region is lined with 3 types of cells (Figure *8*). Approximately $\frac{2}{3}$ of the duct are covered with sac-shaped cells which average 37 μm in height and which resemble mucus secreting cells in other parts of the reproductive system. These cells stain with methyl green. Compressed between these sac-shaped cells are wedge-shaped interstitial cells. These 2 cell types, interstitial and mucus secreting, comprise the post-ampullar gland, the function of which will be discussed later.

The remaining $\frac{1}{3}$ of the first region of the post-ampullar duct is lined with columnar ciliated epithelium and the whole duct is bounded by a layer of connective tissue (Figure *8*).

The anterior bend of the post-ampullar duct over the albumen gland marks the second histological region of the duct. After the bend, the columnar ciliated cells are replaced entirely by the post-ampullar gland cells.

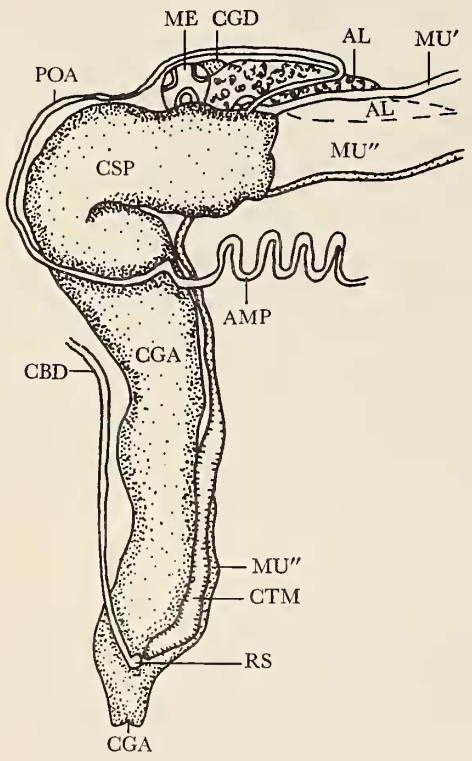

Figure 7

Ventral view of female gland mass and common genital duct showing bend in post-ampullar duct and disappearance of the duct between the albumen gland and common genital duct. Dashed line indicates continuation of the albumen gland between the two lobes of the mucous gland approximately × 10

AL – Albumen gland
AMP – Ampulla proper
CGA – Common genital aperture
CBD – Bursa copulatrix duct
CGD – Common genital duct
CSP – Common genital duct, comma-shaped portion
CTM – Connective tissue membrane
ME – Membrane gland
MU' – Mucous gland, primary lobe
MU" – Mucous gland, secondary lobe
POA – Post-ampullar duct
RS – Bursa copulatrix seminal reservoir

Pallial Gonoduct

All reproductive structures located between the end of the coelomic gonoduct at the fertilization chamber and the common genital aperture comprise the pallial gonoduct (Beeman, 1970). In *Bulla gouldiana* this area includes the complex female gland mass, the common genital duct, the fertilization chamber, and the copulatory bursa.

Female gland mass: The female gland mass forms the bulk of the reproductive structures and consists of the albumen, membrane, and mucous glands, and the oviducal gland which, although a part of the female gland

Explanation of Figures *8*, *9*, *12* to *14*

Figure *8*: Longitudinal section of post-ampullar duct showing the post-ampullar gland and strip of ciliated cells × 150
Figure *9*: Longitudinal section through membrane gland revealing twisting of gland and oviducal groove × 40
Figure *12*: Cross-section through seminal receptacle showing sperm heads in close association with epithelium, and sperm tails projecting into the lumen × 130
Figure *13*: Cross-section of penial sheath and penis. Widely and closely spaced columnar cells are illustrated × 40
Figure *14*: Cross-section through prostate gland showing muscular wall, glandular cells and secretion in the lumen of the gland × 40

AL – Albumen gland
CC – Columnar ciliated cell
CSC – Closely spaced columnar cell
OGR – Oviducal groove
PE – Penis
POAG – Post-ampullar gland
PR – Prostate gland
PRS – Prostatic secretions
PS – Penial sheath
SC – Secretory cell
SP – Spermatozoa
SR – Seminal receptacle

Figure 8

Figure 9

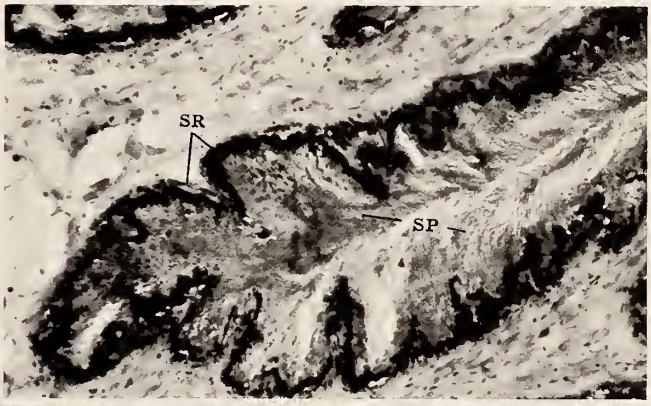

Figure 12

Figure 13

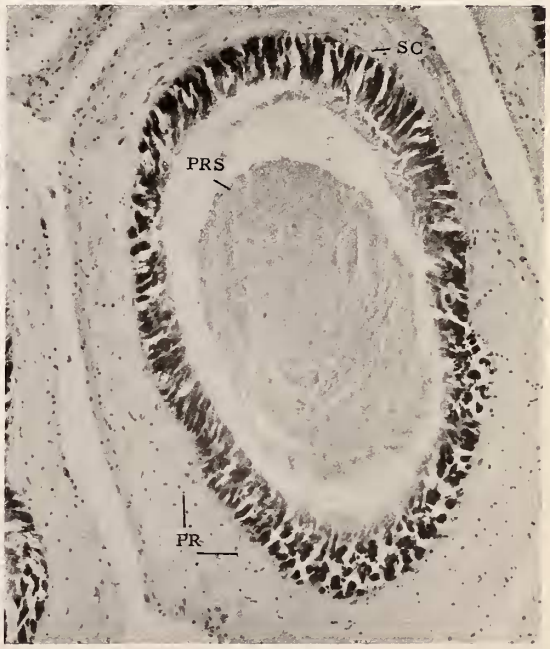

Figure 14

mass, is contained within the muscular walls of the common genital duct (Figures 2, 3, 7).

The anatomy of each gland will be discussed below, but some of the intricate connections and the route followed by the ova during oviposition will be described in the functional morphology section. The structure of the oviducal gland will be discussed in the functional morphology section. The structure of the oviducal gland will be described with the common genital duct.

Albumen gland: The albumen gland is located centrally in the female gland mass (Figures 2, 3, 7). The posterior section of the gland is wedged between the primary and secondary lobes of the mucous gland while the anterior region is bordered by the membrane gland and common genital duct (Figure 7). The left edge of the albumen gland partially surrounds the post-ampullar duct as this duct enters the female gland mass.

The albumen gland cells are arranged around small collecting ductules that empty into the larger main albumen gland duct. Anteriorly, the main duct is lined with large columnar ciliated cells similar to those lining the first convolution of the membrane gland. The albumen gland does not stain with methyl green.

Membrane gland: The membrane gland appears as a small projection off the anterior end of the albumen gland (Figures 2, 3, 7). In fresh material it was seen that the gland is a transparent, ciliated, circuitous tube.

Methyl green staining showed that the membrane gland originates in the central region of the female gland mass. The glandular tube twists several times before it becomes confluent with the oviducal gland at the origin of the common genital duct (Figures 9, 10). A small strip of tissue throughout the windings of the membrane gland did not stain with methyl green.

Histological sections showed that the membrane gland originates at the fertilization chamber, which is located centrally within the female gland mass between the albumen and membrane glands and common genital duct origin. The gland leads in a lateral direction from the narrow opening on the right side of the fertilization chamber, and is lined in this region with columnar ciliated cells. These cells comprise the oviducal groove and correspond in position to the strip of tissue in the gland that did not stain with methyl green. The remaining $\frac{2}{3}$ of the tubular duct are lined with sac-shaped cells and interstitial cells similar to those described for the post-ampullar gland (Figure 9).

Mucous gland: The yellow and semitranslucent mucous gland is the largest of the female gland structures (Figures 2, 3). Its center portion parallels the intestine and

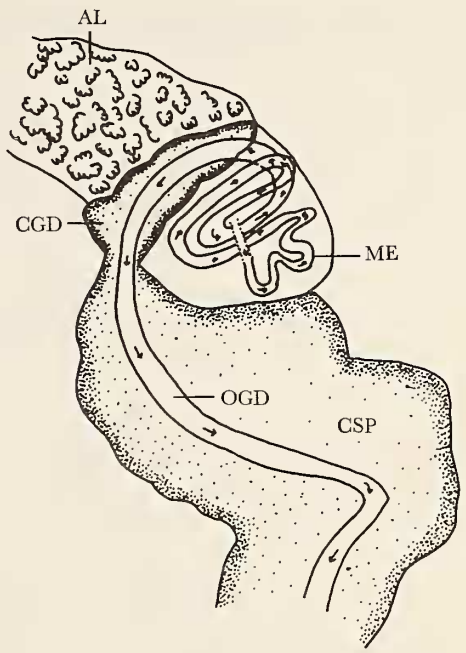

Figure 10

Schematic diagram showing path of the membrane and oviducal gland. Arrows indicate direction of ciliary current

AL – Albumen gland OGD – Oviducal gland
ME – Membrane gland CGD – Common genital duct
CSP – Common genital duct, comma-shaped portion

passes to the left of the albumen and membrane glands. In this region, the mucous gland encloses the posterior tip of the albumen gland between its 2 lobes (Figure 7). Anteriorly, beyond the membrane gland, the mucous gland bends 90° and follows the common genital duct toward the common genital aperture. In this region the mucous gland lies dorsally over the common genital duct. Posteriorly, a short distance after the enclosure of the albumen gland, the mucous gland again turns 90° and follows the intestine toward the anus where the gland tapers and ends.

The mucous gland consists of a primary and a secondary lobe. The primary lobe is anatomically continuous with the oviducal gland and originates where the oviducal gland surfaces dorsally on the common genital duct and makes a 180° loop near the common genital aperture (Figure 3). The narrow primary lobe continues posteriorly and at the tip of the mucous gland near the anus the primary lobe becomes continuous with the secondary lobe. The secondary lobe continues anteriorly and as the lobe

approaches the common genital aperture, the gland twists ventrally and becomes confluent with the common genital duct prior to the common genital aperture.

The cellular organization of the primary lobe is similar to that of the membrane gland and consists of a ciliated oviducal groove and sac-like cells which alternate with ciliated cells. In the secondary lobe large sac-like cells similar to those found in the primary lobe are arranged in a "C" configuration around the lumen. The open end of the configuration is secured by connective tissue.

Both lobes of the mucous gland stain darkly with methyl green, except in the region of the oviducal groove of the primary lobe and the connective tissue closure of the secondary lobe.

Common genital duct: The common genital duct is composed of exogenous and endogenous sperm-containing structures and an oviducal pathway. This muscular duct rests ventrally in the posterior body cavity parallel with the muscular division between the two body sections. The duct originates between the albumen gland and membrane gland as a small triangular area (Figures 3, 7). From this region the duct extends ventrally and widens to form a large comma-shaped section that is tucked under the dorsal edge of the intestine and digestive gland. The duct then bends 90°, narrows, and travels toward the right side of the body to the common genital aperture. The narrow region of the duct is covered dorsally by the primary and secondary lobes of the mucous gland.

The common genital duct consists of several ciliated tracts formed by interlocking grooves and folds of the duct wall (Figure 11). These tracts include the spermoviduct, copulatory duct, seminal receptacle, and the seminal receptacle duct.

The spermoviduct consists of the oviducal gland, oviducal groove and the spermoviducal fold (Figure 11). Methyl green staining showed the oviducal gland to be continuous with the last dorsal folding of the membrane gland (Figure 10). This origin of the duct is in the area corresponding to the tip of the triangular region of the common genital duct (Figures 3, 7).

Methyl green staining also revealed the course of the oviducal gland throughout the rest of the common genital duct. The oviducal gland extends ventrally into the comma-shaped region of the common genital duct. Midway into the narrow region of the duct, the oviducal gland completes 2 clockwise turns, and proceeds toward the common genital aperture. The oviducal gland separates from the rest of the spermoviduct prior to the aperture and bulges through the dorsal surface of the common genital duct where it becomes confluent with the primary lobe of the mucous gland. The oviducal gland is lined

Figure 11

Schematic diagram of a cross-section through the common genital duct in the narrow region before the first loop of the oviducal gland. The relationship of the spermoviduct, copulatory duct, seminal receptacle, and seminal receptacle duct is illustrated

CD – Copulatory duct
CT – Connective tissue
M – Muscle
OGD – Oviducal gland
OGR – Oviducal groove
SO – Spermoviduct
SOF – Spermoviducal fold
SR – Seminal receptacle
SRD – Seminal receptacle duct

with sac-shaped cells interspersed with wedge-shaped ciliated cells similar to the cells found in the membrane, mucous, and post-ampullar glands.

The spermoviducal fold separates the oviducal gland and groove from the copulatory duct, and consists of an outfolding of the muscular wall of the common genital duct (Figure 11). The fold originates with the oviducal gland and first appears as a small projection from the right wall of the last dorsal convolution of the membrane gland. The spermoviducal fold accompanies the oviducal gland and groove through the comma-shaped region and the first part of the narrow region of the common genital duct. The fold and oviducal portion separate at the first clock-wise turn of the oviducal gland. The spermoviducal fold retains a right dorso-lateral position in the common genital duct, and begins to develop folds on its dorsal surface that interlock with grooves developing on the opposite wall to the common genital duct. Near the common genital aperture the folds and grooves occupy the right dorso-lateral wall of the common genital duct, and become

continuous with the external genital groove (Figures 2, 3).

The copulatory duct begins at the common genital aperture, extends through the common genital duct, and ends at the origin of the spermoviduct (Figure 11). At the common genital aperture the copulatory duct is located on the ventral floor of the common genital duct, and forms a pocket on the right side of the duct. This pocket is maintained through the oviducal gland loops and follows the same path through the common genital duct as the rest of the spermoviduct. The copulatory duct ends where the spermoviducal fold projects from the right wall of the last membrane gland loop.

The copulatory duct is lined with cuboidal or columnar cells which are replaced with the cuboidal seminal receptacle epithelium as the duct leads into the seminal receptacle.

The seminal receptacle, or spermatocyst, is an outpocketing of the copulatory duct. This structure is a separate organ in many opisthobranchs (Beeman, 1970; Thompson & Bebbington, 1969) but is contained within the walls of the common genital duct in *Bulla gouldiana*. The receptacle consists of a large lumen with folded walls that form many pockets around the periphery (Figure 11). The receptacle first appears in the narrow region of the common genital duct between the two twists of the oviducal gland and groove is concentrated in the comma-shaped region. As the common genital duct narrows toward its origin, the pocketed areas become less extensive and disappear in the triangular region.

Histological sections showed that the receptacle is lined with columnar to cuboidal epithelium. Sperm are frequently seen in the receptacle with their heads in close association with the epithelial cells and their tails projecting into the lumen (Figure *12*).

Fertilization chamber: The fertilization chamber is frequently called the focal point of the entire reproductive system (Beeman, 1970). In *Bulla gouldiana* the fertilization chamber occurs centrally in the albumen-membrane gland complex, and is a crossroad where several ducts end and others begin. The post-ampullar duct enters the area dorso-laterally on the left side, and on the opposite side the chamber opens into the membrane gland. The seminal receptacle duct joins the chamber ventrally.

Copulatory bursa: The copulatory bursa is a thin-walled sac containing a brownish-red material, the volume of which is variable. The bursa is located dorsally in the posterior body section, on the left side of the heart, and directly beneath the thin mantle covering (Figures 2, 3). The copulatory bursa duct leads from the left medial side of the bursa, and lies parallel and ventral to the common genital duct (Figures 3, 7). The duct opens ventrally into the common genital duct just prior to the merger of the secondary lobe of the mucous gland with the genital duct. At the entrance of the copulatory bursa duct into the common genital duct, the bursa forms a small nodule, or seminal reservoir (Tchang-Si, 1934).

The wall of the copulatory bursa consists of an outer cuboidal layer, a middle connective tissue layer, and an inner closely packed layer of columnar cells. Live and fixed preparations of the contents of the bursa revealed that in addition to the brownish-red secretion, the sac contains spermatozoa and oocytes in various stages of disintegration.

The copulatory bursa duct consists of an outer layer of squamous cells and a middle layer of muscle and connective tissue. The lumen of the duct is lined with ciliated columnar cells about 17 μm in height. The seminal reservoir arising from the duct at its entrance into the common genital duct is lined by squamous cells. The lumen of the reservoir contains closely packed sperm.

External Genital Groove

The external genital groove begins at the common genital aperture and travels anteriorly along the right side of the body to the penial aperture (Figures 2, 3). The groove may be considered an external extension of the pallial gonoduct (Beeman, 1970), and consists of 3 flaps of tissue formed by outward extensions of the body wall. The lower flap covers an inner, smaller flap that interlocks with the upper flap to form a channel.

The flaps of the external genital groove consist of muscle and connective tissue, and their facing surfaces are lined with columnar cells. The cells lining the middle and upper flaps are ciliated and the cellular composition of the duct is constant throughout its entire length.

Copulatory Apparatus

The copulatory apparatus consists of a penial sheath, penial retractor muscle, penis, and prostate gland. The penial sheath is invaginated into the anterior body section and is often twisted around the esophagus (Figures 2, 3). Anatomically, the sheath is divided into neck and bulb regions. The neck region begins at the penial aperture, located beneath the right side of the cephalic shield and right eyespot. The penial retractor muscle inserts into the musculature of the sheath in the neck area and then extends ventrally to the body wall where it originates. Distal to the penial aperture and following the neck region the penial sheath widens, and forms a bulb with a caecum projecting from the end (Figure 3).

Coiled within the penial sheath are the penis and prostate gland. The tubular penis begins at the penial aperture and extends through the neck of the sheath, and is coiled several times in this region. Histological sections of the penis showed that this organ is a muscular tube, and that most of the lumen of the tube is lined with unusually widely spaced columnar cells, which may represent an internal extension of the external genital groove as occurs in other opisthobranchs (Beeman, 1970) (Figure *13*).

The prostate gland is continuous with the penis. The gland is coiled many times in the bulbous end of the penial sheath and terminates as the caecum that projects from the top of the bulb (Figure 3). The prostate is surrounded by an outer muscular layer and its lumen is lined with columnar glandular cells (Figure *14*).

Functional Morphology

Endogenous sperm: Sperm produced within the animal leave the acini of the ovotestis after maturation and pass to the ampulla proper where they are stored. The pre-ampullar ducts connecting the acini and ampulla proper are white and full of mature sperm, particularly during the reproductive season. However, live and histological preparations revealed that sperm are present in the ampulla proper at all times of the year, and are clumped as they are in the acini (Figure *6*).

Prior to copulation the endogenous sperm are transferred from the ampulla proper to the penis (Figure 15). The sperm pass from the ampulla proper, probably aided by the ventral ciliated strip of cells, to the post-ampullar duct. In the post-ampulla, ciliary currents created by the interstitial and columnar cells carry the sperm toward the fertilization chamber. While passing through the post-ampullar duct the bundles of sperm gain a common orientation and are held together as a coherent mass.

Upon reaching the fertilization chamber, the endogenous sperm enter the common genital duct and pass into the spermoviduct. In the spermoviduct the seminal strand is conveyed toward the common genital aperture along the top of the spermoviducal fold, and in the grooves formed on the wall of the common genital duct located opposite to the fold. Sperm were seen in these grooves near the common genital aperture.

Endogenous sperm are transported in the external genital groove to the penial aperture. At the aperture, sperm enter the penis and are moved to the prostate gland where they are stored for several days. Sperm contained within the copulatory apparatus are not clumped together as they are in the ampulla proper and acini of the ovotestis.

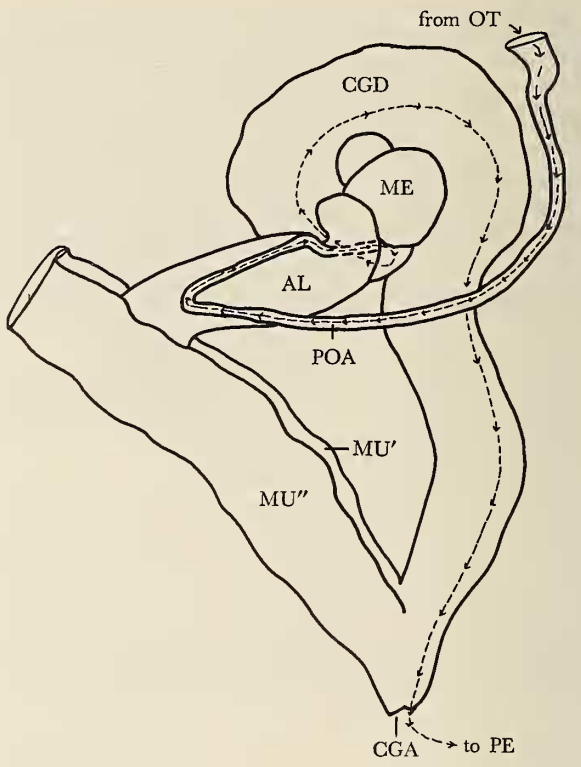

Figure 15

Functional diagram of the female gland mass and the common genital duct following the path of the endogenous sperm from the ampulla proper to the common genital aperture

AL – Albumen gland
ME – Membrane gland
MU' – Mucous gland, primary lobe
MU" – Mucous gland, secondary lobe
POA – Post-ampullar duct
CGA – Common genital aperture
CGD – Common genital duct
OT – Ovotestis
PE – Penis

Copulation: Copulation was observed in the field at night during the spring and summer. Two *Bulla gouldiana* mate with their right sides together, and the penis of the individual assuming the male role is inserted through the common genital aperture and into the copulatory duct of the snail assuming the female role. Reciprocal copulation was not observed.

In the field several copulating pairs were plunged into liquid nitrogen and later dissected in the laboratory in copula. In the female *Bulla gouldiana* the extended penis of the male and the surrounding penial sheath were seen in the copulatory duct.

During live dissections, stimulation of the area around the penial aperture caused eversion of the penis and a white material containing sperm emptied from the duct. Dissection also showed that the whole sheath is capable of peristaltic movement, and this movement was seen occurring while the contents were being exuded. This peristaltic mechanism may transfer the sperm when the penis is placed in the copulatory duct of the individual assuming the female role.

Copulating individuals were separated and the penis was found to be firmly anchored in the copulatory duct. The slight twisting of the path of the copulatory duct within the common genital duct probably helps to secure the penis during copulation.

Exogenous sperm: Exogenous sperm, or spermatozoa received from another animal during copulation, are deposited in the copulatory duct and then moved to the seminal receptacle where they are stored with their heads oriented toward the wall and their tails projecting into the lumen (Figure *12*). Excess sperm received during copulation are probably directed toward the copulatory bursa. Dissection showed the cilia of the copulatory bursa duct forcing sperm from the common genital duct toward the bursa.

Oviposition: Dissection and histological examination of animals frozen in liquid nitrogen revealed the path of the oocytes and the formation of the gelatinous egg-string during oviposition. With the proper stimulus, possibly nervous or hormonal, the oocytes exit en masse from the acini into the pre-ampullar ducts. The oocytes then pass into the ampulla proper, and are conveyed by the ventral ciliated strip of cells through the endogenous sperm present in the duct toward the post-ampullar duct (Figure 16).

In the post-ampulla the oocytes continue toward the fertilization chamber. The post-ampulla was seen filled with oocytes, and the duct was distended as it passed over the membrane and albumen glands. After the bend, the duct narrows and fewer oocytes are able to occupy the lumen. Only 1 to 6 ova were observed in the lumen in the post-ampullar duct in the region prior to the fertilization chamber.

Fertilization occurs in the fertilization chamber, where the post-ampullar and seminal receptacle ducts converge (Figure 16). Sperm were seen in the seminal receptacle duct leading to the fertilization chamber, and in dissections the muscular common genital duct exhibited peristaltic movement. These muscular actions could help transport exogenous sperm to the fertilization area, along with the activity of the ciliated cells of the seminal receptacle duct.

From the fertilization chamber, the fertilized group of ova passes into the membrane gland and simultaneously receives a layer of albumen. In the membrane gland the ova pass along the oviducal groove as the first layer of mucus, or capsule, is secreted around them by the large sac-shaped secretory cells.

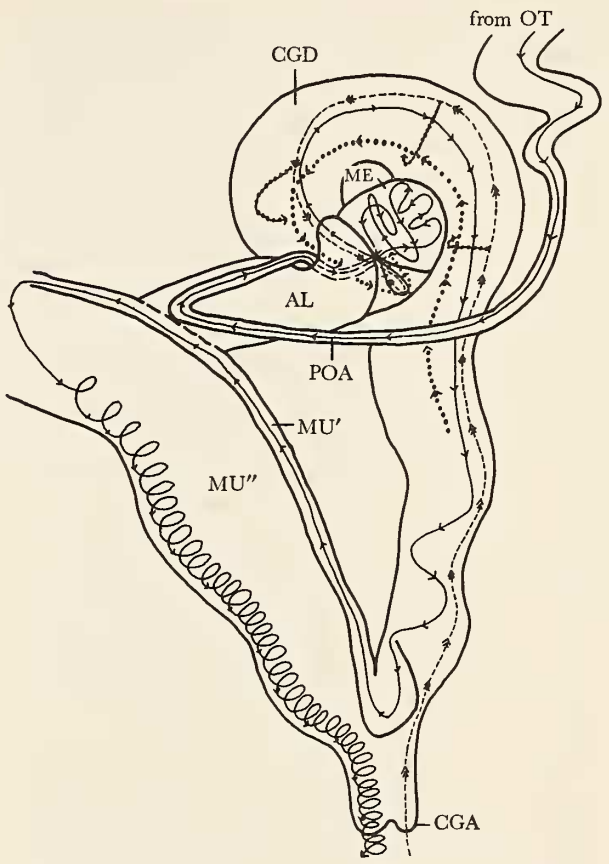

Figure 16

Functional diagram of the female gland mass and common genital duct showing routes followed by exogenous spermatozoa and oocytes during oviposition

AL – Albumen gland MU' – Mucous gland, primary lobe
CGA – Common genital aperture OT – Ovotestis
CGD – Common genital duct POA – Post-ampullar duct
ME – Membrane gland MU" – Mucous gland, secondary lobe

→– – –→ Path of endogenous sperm through reproductive system
»– – –» Path of exogenous sperm received at copulation
»···»···» Path of exogenous sperm after storage in seminal receptacle in route to fertilization chamber
Path of egg-string during oviposition
→——→ Oocyte pathway from ovotestis to mucous gland